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Use of Laboratory Tests for Immune Biomarkers in Environmental Health Studies Concerned with Exposure to Indoor Air Pollutants

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The immune system is likely to be involved in some of the health effects caused by certain indoor air exposures, and immune biomarkers can help determine which exposures and health effects have important immune components. However, the lack of standardized laboratory tests for most human immune markers and the many confounding variables that can influence them makes interpretation of results for exposure and disease end points uncertain. This paper presents an overview of the immune system and the considerations involved in using tests for immune markers in clinical epidemiology studies, particularly those concerned with indoor air exposures. Careful study design, well-characterized laboratory methods, and rigorous documentation of exposure status are required to determine the predictive value of such tests. Clinical tests currently available for some immune markers could help identify and characterize both irritative and hypersensitivity reactions to indoor air pollutants. Newer tests developed in research settings might provide more incisive indicators of immune status that could help identify exposure, susceptibility, or preclinical disease states, but their methodologies must be refined and tested in multicenter studies before they can be used reliably in public health applications.

Introduction

The host defense system of mammals is a complex network of cells and biochemical mediators responsible for repairing injured tissue, identifying and removing foreign substances, destroying or containing infectious agents, and in some cases eradicating cancer cells. The host defense system includes both innate (or nonspecific) mechanisms of immunity and acquired (or specific) mechanisms of immunity. In this review, the term "immune system" refers to all components of both innate and acquired immunity, as their components and activities are often intertwined.

Evidence accumulated over the last several years has shown that indoor air pollution is a major source of exposure to environmental chemicals (*1–4*). Environmental pollutants could potentially lead to adverse health consequences through interaction with the immune system in several different ways. As toxicants, they could damage parts of the immune system and impair host defense. As foreign substances (xenobiotics), they could evoke inappropriate responses or intensify normal responses to the point that certain immune-mediated functions become pathological rather than protective. Pollutants could also have indirect effects on the immune system by influencing other organ systems, particularly the neuroendocrine system.

Alternatively, some pollutants could interact with the immune system in ways that would not cause adverse health effects, and others could fail to influence the immune system in any way at all. These various possibilities can be addressed only through longitudinal clinical epidemiology studies that combine rigorous exposure and health assessments with standardized laboratory measurements of the cells and mediators of the immune systems, so-called immune biomarkers. This article presents a brief review of the use of immune biomarkers in such clinical epidemiologic studies, with emphasis on immune physiology and laboratory methods.

Immune System and Environmental Antigens

A number of articles and textbooks provide excellent descriptions of the immune system (*5–7*), and only a brief overview will be given here.

Perhaps the most fundamental characteristic of the immune system is its ability to recognize and destroy foreign material. This activity is exemplified by the pulmonary alveolar macrophage, a larger mobile cell found in the lung sacs that avidly ingests (phagocytizes) foreign material, including bacteria and air pollutants. The macrophage attempts to digest and process ingested material with powerful enzymes in an acid environment. In the case of bacteria, this response can destroy the infectious agent and prevent disease. However, if the ingested material is a particular form of asbestos microfiber, the macrophage itself can be damaged, and lung tissue can be destroyed by the release of its digestive agents. Thus, the normal function of the macrophage

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can prevent or induce disease, depending on the type and dose of material to which it is exposed.

Cells of the Immune System

Most of the several different types of cells that constitute the immune system spend at least part of their lifetime in the peripheral blood, where they make up the white blood cells or leukocytes. The major types of leukocytes are lymphocytes, monocytes, and granulocytes.

Lymphocytes (B-cells and T-cells) are the specific recognition cells of the immune system. Each family (clone) of lymphocytes has unique recognition molecules on its surface, and if lymphocytes are activated by recognizing a foreign protein (antigen) presented by a macrophage (or similar cell), a specific immune response is initiated. Activated lymphocytes engage in a variety of host defense functions, such as producing antibody (B-cells) and killing virus-infected cells (T-cells).

Monocytes become macrophages when they emigrate from the blood. They are distributed throughout many tissues including the lung, liver, skin, brain, and bone marrow. Their innate activities of phagocytosis and digestion are nonspecific, but they become part of the specific immune response when they present processed fragments of foreign protein to lymphocytes.

Granulocytes are important auxiliary cells with activities that are critical to host defense and may also contribute to disease processes. Neutrophils, like macrophages, are avid phagocytes, but they are short-lived and less versatile. Mast cells, basophils, and eosinophils are involved with immunity to larger parasites such as worms, and they are the primary participants in the allergic responses to pollens, foods, and other substances. They also appear to be involved with inflammatory reactions to certain toxic and sensitizing chemical exposures (*8–10*).

Chemical Mediators of the Immune System

Many of the defense and regulatory functions of the immune system are conducted by chemical mediators released from its cells. The only antigen-specific mediators are the antibodies secreted by stimulated B-lymphocytes. Antibodies (also called immunoglobulins [Ig]) are composed of several major classes with different functional capacities. IgM and IgG antibodies are the most general-purpose types; they facilitate phagocytosis, antigen clearance and destruction of parasites. IgA antibodies are secreted at the mucous membranes, where they help prevent attachment and invasion by the billions of bacteria and viruses that come in contact with these surface tissues. IgE antibodies are bound to the outer membrane of mast cells and basophils, where they serve as the recognition molecules for antigens that cause allergic reactions like hay fever.

Cytokines are extremely potent chemical messengers that activate or suppress the functional and metabolic activities of target cell populations that express the appropriate receptors. About a dozen immune cytokines called interleukins have been well-characterized. Interleukin-1 (IL-1) and interleukin-2 (IL-2) play especially pivotal roles in the overall immune response.

Complement is one of several plasma proteins involved with host defense. It is actually a cascading system of different protein molecules that can be activated by antigen–antibody complexes, blood-clotting proteins, and other substances. Complement activation products have a number of activities, including chemotaxis, clearance, and destruction of cells.

Several small molecules are important immune mediators. They include different lipid-derived chemicals (such as prostaglandins) that have a wide variety of effects on many different tissues, such as the activation or suppression of immune cells and the dilation or constriction of blood vessels and airways. Histamine, which is stored in the granules of mast cells and basophils, causes dilation and leakage in small blood vessels and has effects on immune cells and other tissues; it is responsible for many of the symptoms of allergy.

Several other chemical mediators have influences on cells of the immune system, although they are not as central to its function. These include catecholamines (such as adrenalin), endorphins, insulin, transferrin, and others.

Antigens, Haptens, and Allergens

An antigen is a substance that sensitizes an animal to evoke a specific immune response upon subsequent exposure. The presence of a specific immune response may be demonstrated by immunochemical assays that detect antibodies specifically bound to the antigen, by cellular function assays that show lymphocytes reacting specifically to the antigen, or by specific hypersensitivity reactions in tissues exposed to the antigen (e.g., skin tests).

In order for small molecules (including almost all xenobiotic chemicals) to be antigenic, they must first combine with tissue proteins, creating a complex that the immune system recognizes as foreign. The chemical is called a hapten and the protein to which it is bound is called a carrier. Hapten–carrier complexes can be formed when reactive chemicals (e.g., isocyanates) form covalent bonds with tissue proteins or when inert chemicals (e.g., nickel compounds) are tightly bound to proteins through chelation.

Antigens such as ragweed pollen that evoke immediate hypersensitivity responses are frequently called "allergens" because of their association with the clinical symptoms of allergy. The factors that make an antigen behave as an allergen depend on the immune response of the individual and (presumably) not on the chemical nature of the antigen itself. Allergens do in fact generate other immune responses in addition to those associated with immediate hypersensitivity.

Specificity of Antibodies

A normal "specific" antibody response includes a mixture of tens, hundreds, or thousands of antibodies with different specificities. The nature of this mixture depends on the dynamic response of living cells (B-lymphocytes), and it changes as the animal ages and as each immune response "matures" over time. It also can vary enormously between different individuals. Individual variance in immune reactivity is especially notable with the limited responses evoked by the small hapten molecules.

For both technical and biological reasons, assays that measure only antibody binding do not by themselves establish the presence of antigen-specific antibodies. Specificity must be documented by competitive binding assays and, preferably, by purification (or at least concentration) of the specific antibody population.

Inflammation, Irritation, and Hypersensitivity

Inflammation, characterized by redness, swelling, heat, and pain, is the tissue response to injury and invasion by foreign material. It begins as a reaction in the microscopic blood vessels, which enlarge and open their walls allowing cells and mediators to escape from the blood and enter the tissue. An inflammatory response may be evoked by simple skin injury such as a scratch, without any antigen stimulating a specific immune response. These simple inflammatory responses are often called irritant reactions. However, if a previously encountered antigen enters the tissue, the inflammatory response may be greatly accelerated by specific lymphocytes or antibodies, and an immune-mediated hypersensitivity reaction will occur. Inflammation due to hypersensitivity reactions may be immediate (as in allergies), acute (as in serum sickness), or delayed (as in tuberculosis or beryllium lung disease).

Most inflamed tissue contains a mixture of irritative reactions and hypersensitivity responses in their various phases. The distinctions may be difficult to discern, especially in the mucosal tissues where inflammation is chronic (see below). In addition, irritant responses may augment hypersensitivity reactions through nonspecific "adjuvant effects," such as those attributed to air pollutants including diesel fly ash (*11*), ozone (*12*), and sulfur dioxide (*13*). Both types of response may contribute to symptoms of disease, but hypersensitivity responses, because of their greater magnitude, are more likely to result in significant tissue damage.

Immune Function at the Surface Tissues of the Body

Because most tissue injuries and potential sites of invasion by parasites occur along the surface tissues of the organism, immune function is especially well developed in these areas (*14,15*). The two types of surface tissues are the skin and the mucosa, the latter found in the resriratory, gastrointestinal, and genitourinary systems. Both the skin and the mucosa contain specialized cells and tissues that promote host defense. Mucosal surfaces in particular are characterized by specialized "goblet" cells that secrete mucous, and they are chronically inhabited by immune cells responding to the constant barrage of environmental irritants and antigens.

Disorders of the Immune System

Three general types of disorders of the immune system may have adverse health consequences: immune deficiencies, inappropriate immune reactivities, and unregulated immune proliferation.

Immune deficiency disorders, in which the immune system fails to mount adequate protective responses against infection or certain forms of cancer, may be caused by several different types of immunosuppressive agents. Depending on the nature of the deficiency, the health consequences can range from almost undetectable (such as increases in the incidence of mild infections) to life-threatening (such as overwhelming sepsis). High exposures to solvent vapors such as benzene and trinitrotoluene may cause aplastic anemia, which is immunosuppressive since the bone marrow precursors for all types of blood cells, including leukocytes, are destroyed. In addition, benzene may have selective suppressive effects on lymphocytes (*16*).

Immune reactive disorders are those in which immune activity damages host tissues due to inappropriate or poorly regulated responses. Again, depending on their cause and nature, such disorders can be very mild or very severe. Common allergies are caused by inappropriate immediate hypersensitivity responses that release histamine and lipid-derived mediators. These allergic reactions are often directed against airborne antigens and may contribute to the pathogenesis of asthma. Autoimmune diseases are debilitating immune reactive disorders in which the immune system reacts against its own tissues. Autoimmune reactions can damage the skin, liver, kidneys, various glands, joints and other tissues, leading to diseases such as rheumatoid arthritis, systemic lupus erythematosis, and some types of diabetes. Antibodies associated with autoimmune reactions react with self-proteins in particular tissues or cell components. Exposure to vinyl chloride, a volatile toxicant and carcinogen, can cause a severe autoimmunelike illness in humans (*17*).

Immune proliferative disorders involve the unregulated growth of one family among the immune cells, leading to leukemia, lymphoma, and similar cancers of the immune tissue. Exposure to high concentrations of benzene vapors greatly increases the risk of developing certain leukemias in humans (*16*).

Nervous System, Stress, and Conditioning of the Immune Response

The overall immune response is influenced by many systemic factors, including the nervous system, both indirectly (through neuroendocrine influences) and directly. Direct interactions between the nervous system and the immune system appear to be especially important in the mucosal surfaces, where nerve cells and mast cells may communicate constantly (*18*). One consequence of these interactions is that stress is an important modulator of immune function and one of the most difficult variables to evaluate.

Recent studies in experimental animals have shown that changes in immune status and function can be elicited by a neural stimulus to which the animals have been previously conditioned. Both suppressive and reactive changes have been observed to conditioning stimuli such as taste, odor, and audiovisual cues (*19,20*). Therefore, under some circumstances, the perception of exposure may trigger the same mechanisms that actual exposure triggers, causing the appearance of an immune reaction (such as allergy) in a conditioned animal without any actual exposure to antigenic material.

Laboratory Assessment of the Immune System

During the last two decades, remarkable advances in technology and in our understanding of the chemical and cellular constituents of the immune system have allowed the development of many assays that evaluate different aspects of immune status or function, collectively referred to as immune biomarkers. Historically, immune biomarkers have been extremely useful in detecting exposure to infectious organisms, in characterizing the defects that cause immune deficiencies, and in delineating the pathogenesis of immune-mediated diseases. In these situations, laboratory tests can be applied to targeted populations, often associated with clinical illness, with some evidence of the immune system as a primary participant in the pathologic processes.

The use of immune biomarkers in environmental health studies to establish (or attempt to rule out) the potential for long-term health effects due to toxicant exposures is an entirely different application. A major difficulty underlying the use of laboratory tests in such studies is the decline in positive predictive value when the prevalence of exposure or disease is low. Additional problems arise with immune biomarkers because of their wide range of normal variability, the influence of many confounding factors including neuroendocrine effects, and, most fundamentally, from the dearth of standardized, well-characterized laboratory methods. Finally, the lack of precise exposure measurements is a major impediment toward documenting any relationship between toxicants, biomarker changes, and health effects.

General Principles for Using Immune Biomarker Assays

Although the proper use of tests for immune biomarkers may be helpful in environmental health studies, their improper use can create confusion and undermine public health efforts. To address these concerns, the Centers for Disease Control (CDC) and the Agency for Toxic Substances and Disease Registry (ATSDR) convened a subcommittee on biomarkers of organ damage and dysfunction to develop proposed guidelines for the use of biomarker tests in health assessment studies conducted at Superfund sites. The following points are adapted from their final report *(21)*.

1. The public health goal of using tests for biomarkers is to be able to inform a population that the results do or do not suggest the possibility of increased morbidity or mortality caused by toxicant exposure. Therefore, test results should be interpretable in the context of health effects and exposures. Toward this goal, normative data obtained from standardized laboratory methods are essential in assessing the sensitivity and specificity of a test for health or exposure end points. Exposure end points should be documented by measured levels of toxicants whenever possible.
2. When tests with unknown sensitivity and specificity are used in health surveillance studies, they should be evaluated under investigative protocols in populations with known exposure or disease end points and compared to tests having the best-characterized sensitivity and specificity for those end points. Before a test is considered to have completed the investigative phases, the biochemical or physical abnormality associated with the biomarker should be identified, and the nature of any disease associations should be determined.
3. Tests for biomarkers should be organized into categories that allow the most cost-effective public health implementation. For the immune system, the subcommittee identified three such categories: a basic panel as a general evaluation of immune status, focused/reflex tests that address particular aspects of immune function, and research tests that require evaluation in defined populations before general use in either of the first two categories. Tests in both the basic panel group and the focused/reflex group must have clinical interpretations for disease endoints when values lie outside established reference ranges. Tests from the basic panel should be included when there is no clear indication of particular health effects or well-defined exposures. Tests from the focused/reflex panel are suggested by particular clinical symptoms, prior laboratory findings, or specific exposures; they may be used individually or to augment the basic panel. Research tests should be used under the auspices of an investigative protocol, as discussed above.

Issues in Laboratory Methodology for Immune Biomarkers

The CDC/ATSDR subcommittee identified the lack of standardized laboratory methods as the most critical area requiring further research and development for the proper use of immune biomarkers in public health studies. Most immunochemical and immunobiological measurements, especially those evaluating cellular parameters, are considerably more complex than simpler biochemical measurements such as enzyme activities. Moreover, different methods for measuring a particular analyte can give entirely different results. Even assays that have been quite informative under controlled experimental conditions in research settings may not prove useful in public health studies.

For both scientific and pragmatic reasons, any laboratory method must be evaluated and implemented successfully in multiple laboratories before it can be useful in public health investigations. Scientifically, the hallmark of a true laboratory finding is its reproducibility, and no laboratory result can be considered factual until it is independently replicated. Pragmatically, public health evaluations often require multisite longitudinal studies in which assays are conducted at different laboratories and at different times. Therefore, any laboratory assay used for public health evaluations, no matter how simple or esoteric, should be continually monitored for its scientific validity through comparisons within and between laboratories.

Applying Immune Biomarkers to Indoor Air Exposure Studies

Beyond the issues of methodology and predictive value, the main considerations for selecting immune marker tests must be the expected types of exposures from indoor pollutants and their potential effects on human health (Table 1). An overview of the major types of tests that might be helpful follows.

Antibodies to Environmental Material

Allergic reactions mediated by antibodies to environmental antigens from microbes, molds, mites, plants, fabric fibers, insects, and mammals may be contributors to many of the symptoms associated with indoor air exposure *(22–25)*. The total serum IgE antibody level may help discriminate exposed or susceptible ("atopic") populations, but the total level does not have good predictive value on an individual basis *(26)*. Tests for antigen-specific IgE antibodies (skin testing or *in vitro* assays) have better predictive value than total IgE for allergic reactions against many inhaled antigens, but sensitization may be found in normals as well as symptomatic patients (W. K. Dolen and P. B. Williams, unpublished data).

Table 1. Some examples of indoor air pollutants that cause health effects related to the immune system.

Effect related to immune system	Examples of causative indoor air pollutants
Inflammation caused by irritant and adjuvant reactions	Respirable particles (smoke dust); volatile or gaseous chemicals (formaldehyde, nitrogen dioxide, solvents)
Inflammation caused by hypersensitivity reactions	Biologically derived antigens (bacteria, molds, plants, mites, insects, pests, pets, livestock, humans); industrial chemicals (beryllium, isocyanates, others in occupational settings)
Autoimmune reactions	Vinyl chloride (occupational settings)
Immune suppression	Benzene, trinitrotoluene (occupational settings)
Neoplastic proliferation	Benzene (occupational settings)
Conditioned neuroendocrine effects on immune function	Dimethylsulfide, trimethylamine (demonstrated only in animal models; may be elicited by other cues)

Detecting antibodies to environmental chemicals is more complicated, partly because of the specificity considerations discussed earlier and partly because of technical problems in developing laboratory assays. In occupational settings, human antibodies have been detected against anhydrides (*27*) and isocyanates (*28*), both reactive chemicals that couple to proteins. Human antibodies to formaldehyde-treated proteins have also been reported (*29,30*), but only in a few cases has their specificity been documented by appropriate inhibition assays, and such assays have not demonstrated specific antibodies in any airborne exposures to formaldehyde (*29*).

Auto-Antibodies

As discussed above, the immune system may react against its own tissues and produce "auto-antibodies." The pathological significance of auto-antibodies depends on their concentration, specificity, avidity, and effector functions. High concentrations (titers) of auto-antibodies are often indicative of active disease processes, whereas low titers (as measured by immunofluorescence microscopy) are generally not associated with obvious clinical end points. Some low-titer auto-antibodies may reflect increased tissue clearance rather than an uncontrolled immune response, and as such they might be useful biologic markers for inflammatory damage caused by irritant responses to airborne chemicals. However, virtually none of the assays for auto-antibodies are sufficiently standardized to measure weak reactions reliably, and reports of low-titer auto-antibodies associated with certain airborne exposures (*30*) require independent verification.

Other Humoral Mediators

The serum proteins involved with inflammatory responses (such as complement) may provide some indication of irritative or immune reactions to air pollutants. For instance, one report found that a small but consistent shift toward higher concentrations of a complement protein was associated with the extent of outdoor air pollution (*31*). The predictive value of this difference was quite low, but the report was well documented. and the assay method was sound. The finding emphasizes both the reality of air pollution effects and the myriad of confounding variables that can influence immune markers.

Other mediators discussed above (e.g., prostaglandins) may be useful as markers of inflammatory or immune processes. Tests for most of these mediators should be considered only for research protocols, as their assays are often poorly standardized and their clinical meaning generally unclear.

Peripheral Blood Cells

In humans, the vast majority of analysis for cellular markers is performed on peripheral blood white cells (leukocytes), almost all of which are in an inactive "resting" state. Other cells that are more indicative of current immune activity (such as those from the spleen and lymph nodes) are simply not available in most human studies. Despite this limitation, analysis of peripheral blood leukocytes can provide important information about immune status.

Complete blood counts (CBCs) give the most basic data about the distribution of peripheral leukocytes. CBCs performed for environmental health studies should include a five-part differential to measure all major types of leukocytes, including eosinophils and basophils (many of the automated blood counters do not distinguish the latter two types from neutrophils). All of these measurements are well-standardized if conducted in accord with good clinical laboratory practice, with the possible exception of basophil and eosinophil counts (*32*). The range of results for these cellular parameters in reference populations is very broad (population confidence values of 20–80%) (*33*), and differences within the "normal range" are not associated with obvious or long-term health effects. However, values well outside the "normal ranges" are very likely to be associated with active disease processes that involve the immune system.

Immune cells (lymphocytes in particular) can be characterized more completely by identifying their surface receptors (or immunophenotype), which reveals information about their lineage and activation state. The method of choice for these tests is immunofluorescence flow cytometry (FC) (*34*). Until recently, these measurements were not well standardized, but in the past 3 years considerable effort has been made to increase the reliability of many assay methods (*35,36*). Because some methodologies are still quite variable and do not give comparable results, the assays used in clinical epidemiology studies must be clearly described for findings to have any real value [for good examples, see Shopp et al. (*37*), and Edwards et al. (*38*)]. Efforts toward uniform calibration (*39*) and multicenter laboratory evaluations (*40*) could increase the amount of biological information obtained by FC, much of which is now discarded. Whether any of this information will be useful in assessing the effects of indoor air exposures on the immune system (such as unregulated proliferation) remains an open question requiring thorough epidemiologic and laboratory investigation; previous reports of "immune dysregulation" caused by airborne exposures (*41*) are not documented well enough to substantiate their conclusions.

Functional assays of immune cells from peripheral blood could in theory provide the most useful information about immune status because they test the ability of resting cells in actually responding to a stimulus. However, these assays are very difficult to standardize and require diligent quality control for reliability even within a laboratory (*37*). A variety of different stimuli and functional response parameters can be evaluated; all must be considered research tests with uncertain predictive value for exposure or health endpoints.

Mucosal Tissue Infiltration by Immune Cells

The most promising approach to cellular assessment for indoor air exposures may lie in examining immune cells from accessible mucosal surfaces such as nasal scrapings. Bronchoalveolar lavage can also provide a source of respiratory mucosal immune cells, but it is an unpleasant procedure and would not detect the modest cell infiltration caused by many irritant responses. Nasal challenge and characterization of the human *in situ* nasal mucosal response to airborne exposures is a critical research needs which is just now being addressed. Early results suggest that local inflammatory reactions are important factors in airborne exposures to toxicants (*42,43*).

Summary

The proper use of tests for immune biomarkers in health assessment studies could help identify effects of indoor air pollutants on the immune system and the risk of consequent health effects, but only if the studies are carefully designed to account for confounding factors and if the laboratory methods are rigorously standardized. At this time, tests for inflammatory and allergic mediators are the most likely to give interpretable information about exposures and effects. However, tests characterizing the cellular responses, particularly local tissue responses to airborne pollutants, will be essential for a complete approach to health assessment and exposure effects.

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REFERENCES

1. Norback, D., Michel, I., and Widstrom, J. Indoor air quality and personal factors related to the sick building syndrome. Scand. J. Work Environ. Health 16: 121–128 (1990).
2. Spengler, J. P. Indoor air pollution. N. Engl. Reg. Allergy Proc. 6: 126–134 (1985).
3. Wallace, L. A., Pellizzari, E. D., Hartwell, T. D., Sparacino, C., Whitmore, R., Sheldon, L., Zelon, H., and Perritt, R. The TEAM (Total Exposure Assessment Methodology) Study: personal exposures to toxic substances in air, drinking water, and breath of 400 residents of New Jersey, North Carolina, and North Dakota. Environ. Res. 43: 290–307 (1987).
4. Lebowitz, M. D. Health effects of indoor air pollutants. Ann. Rev. Public Health 4: 203–221 (1983).
5. Roitt, I. M., Brostoff, J., and Make, D. K. Immunology. C. V. Mosby Company, St. Louis, 1987.
6. Yoshida, S., Golub, M., and Gershwin, M. E. Immunologic aspects of toxicology: premises, not promises. Regul. Toxicol. Pharmacol. 9: 56–80 (1989).
7. Luster, M. I., Blank, J. A., and Dean, J. H. Molecular and cellular basis of chemically induced immunotoxicity. Ann. Rev. Pharmacol. Toxicol. 27: 23–49 (1987).
8. Vogt, R. F., Dannenberg, A. M., Papirmeister, B., and Scofield, B. Pathogenesis of skin lesions caused by sulfur mustard. Fundam. Applied Toxicol. 4: S71–83 (1984).
9. Dvorak, H. F. Cutaneous basophil hypersensitivity. J. Allergy. Clin. Immunol. 58: 229–240 (1976).
10. Belongia, E. A., Hedberg, C. W., Gleich, G. J., White, K. E., Mayeno, A. L., Loegering, D. A., Dunnette, S. L., Pirie, P. L., MacDonald, K. L., and Osterholm, M. T. An investigation of the cause of the eosinophiliamyalgia syndrome associated with tryptophan use. N. Engl. J. Med. 323: 357–365 (1990).
11. Muranaka, M., Suzuki, K., Koizumi, S., and Takafuji, S. Adjuvant activity of diesel-exhaust particulates for the production of IgE antibody in mice. J. Allergy Clin. Immunol. 77: 616–623 (1986).
12. Riedel, F., Kramer, M., Scheibenbogen, C., and Rieger, C. H. Effects of SO_2 exposure on allergic sensitization in the guinea pig. J. Allergy Clin. Immunol. 82: 527–534 (1988).
13. Osebold, J. E., Gershwin, I. F., and Zee, Y. C. Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosol. J. Environ. Pathol. Toxicol. 3: 221–234 (1980).
14. Bienenstock, J., and Gauldie, J. Cellular interactions in lung inflammation and immunity. Postgrad. Med. J. 64(suppl.) 4: 111–119 (1988).
15. Cebra, J. J., Gearhart, P. J., Halsey, L. F., Hurwitz, J. L., and Shahin, R. D. Role of environmental antigens in the ontogeny of the secretory immune response. J. Reticuloendothel. Soc. 28(suppl): 61s–71s (1980).
16. Kipen, H. M., Cody, R. P., Crump, K. S., Allen, B. C., and Goldstein, B. D. Hematologic effects of benzene: a thirty-five year longitudinal study of rubber workers. Toxicol. Ind. Health 4: 411–430 (1988).
17. Ward A. M., Udnoon, S., Watkins, J., Walker, A. E., and Darke, C. S. Immunological mechanisms in the pathogenesis of vinyl chloride disease. Br. Med. J. 1: 936–938 (1976).
18. Bienenstock, J., Tomioka, M., Matsuda, H., Stead, R. H., Quinonez, G., Simon, G. T., Coughlin, M. D., and Denburg, J. A. The role of mast cells in inflammatory processes: evidence for nerve/mast cell interactions. Int. Arch. Allergy Appl. Immunol. 82: 238–243 (1987).
19. Kusnecov, A., King, M. G., and Husband, A. J. Immunomodulation by behavioural conditioning. Biol. Psychol. 28: 25–39 (1989).
20. MacQueen, G., Marshall, J., Perdue, M., Siegel, S., and Bienenstock, J. Pavlovian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. Science 243: 83–85 (1989).
21. Subcommittee on Biomarkers of Organ Damage and Dysfunction of the Centers for Disease Control and Agency for Toxic Substances and Disease Registry. Internal Report on Biomarkers of Organ Damage and Dysfunction for the Renal, Hepatobiliary and Immune Systems. Centers for Disease Control, Biochemistry Branch, Atlanta, GA, 1990.
22. Al-Doory, Y. The indoor airborne fungi. N. Engl. Reg. Allergy Proc. 6: 140–149 (1985).
23. Gravesen, S., Larsen, L., Gyntelberg, F., and Skov, P. Demonstration of microorganisms and dust in schools and offices. Allergy 41: 520–525 (1986).
24. Lowenstein, H., Gravesen, S., Larsen, L., and Schwartz, B. Indoor allergens. J. Allergy Clin. Immunol. 78: 1035–1039 (1986).
25. Baldo, B. A., and Panzani, R. C. Detection of IgE antibodies to a wide range of insect species in subjects with suspected inhalent allergies to insects. Int. Arch. Allergy Appl. Immunol. 85: 278–287 (1988).
26. Barbee, R. A., Balonen, M., Kaltenborn, Lebowitz, M., and Burrows, B. A longitudinal study of serum IgE in a community cohort: correlations with age, sex, smoking and atopic status. J. Allergy Clin. Immunol. 79: 919–927 (1987).
27. Bernstein, D. I., Zeiss, C. R., Wolkonsky, P., Levitz, D., Roberts, M., and Patterson, R. The relationship of total serum IgE and blocking antibody in trimellitic anhydride-induced occupational asthma. J. Allergy Clin. Immunol. 72: 714–719 (1983).
28. Cartier, A., Grammer, L., Malo, J. L., Lagier, F., Ghezzo, H., Harris, K., and Patterson, R. Specific serum antibodies against isocyanates: association with occupational asthma. J. Allergy Clin. Immunol. 84: 507–514 (1989).
29. Grammer, L. C., Harris, K. E., Shaughenessy, M. A., Sparks, P., Ayars, G. H., Altman, L. C., and Patterson, R. Clinical and immunologic evaluation of 37 workers exposed to gaseous formaldehyde. J. Allergy Clin. Immunol. 86: 177–181 (1990).
30. Thrasher, J. D., Broughton, A., and Madison, R. Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. Arch. Environ. Health 45: 217–223 (1990).

31. Stiller-Winkler, R., Kramer, U., Fiedler, E., Ewers, U., and Dolgner, R. C3c concentrations in sera of persons living in areas with different levels of air pollution in North Rhine-Westphalia (Federal Republic of Germany). Environ. Res. 49: 7–19 (1989).
32. National Committee for Clinical Laboratory Standards. Leukocyte Differential Counting. Publication H20-T. NCCLS, Villanova, PA, 1984.
33. U.S. Department of Health and Human Services. Total White Blood Cell Counts for Persons Ages 1–74 Years with Differential Leukocyte Counts for Adults Ages 25–74 Years: United States, 1971–75. DHHS Publication No. (PHS) 82-1670, Washington, DC, 1982.
34. Shapiro, H. Practical Flow Cytometry, 2nd ed. Alan R. Liss, New York, 1988, pp. 282–283.
35. Kidd, P. G., and Vogt, R. F. Evaluation of T-cell subsets during HIV infection and AIDS: a workshop report. Clin. Immunol. Immunopathol. 52: 3–9 (1989).
36. National Committee for Clinical Laboratory Standards. Clinical Applications of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes. Publication H42-P. NCCLS, Villanova, PA, 1990.
37. Shopp, G. M., Edwards, B. S., Coons, T. A., Sweeney, M. H., Vogt, R. G., Ashmore, L. M., Harper, D. J., Lane, K. A., and Nolla, H. A. Laboratory assessment of the immune system in individuals occupationally-exposed to 2,3,7,8-TCDD: test selection, assay methods and quality control in a cross-sectional epidemiologic study. Chemosphere 18: 867–874 (1989).
38. Edwards, B. S., Altobelli, K. K., Nolla, H. A., Harper, D. A., and Hoffman, R. R. Comprehensive quality assessment approach for flow cytometric immunophenotyping of human lymphocytes. Cytometry 10: 433–441 (1989).
39. Vogt, R. F., Cross, G. D., Phillips, D. L., and Henderson, L. O. A modeling system evaluating fluorescein-labeled microbeads as internal standards to calibrate fluorescence intensity on flow cytometers. Cytometry 10: 294–302 (1989).
40. Vogt, R. F., Henderson, L. O., Straight, J. M., and Orti, D. L. Immune biomarkers demonstration project: standardized data for flow aquisition and analysis for cytometric assessment of exposed immune status in toxicant persons. Cytometry (suppl.) 4: 103 (1990).
41. Levin, A. S., and Byers, V. C. Environmental illness: a disorder of immune regulation. State Art. Rev. Occup. Med. 2: 669–681 (1987).
42. Bascom, R., Naclerio, R. M., Fitzgerald, T. K., Kagey-Sobotka, A., and Proud, D. Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. Am. Rev. Respir. Dis. 142: 594–601 (1990).
43. Koren, H. S., Hatch, G. E., and Grahara, D. E. Nasal lavage as a tool in assessing acute inflammation in response to inhaled pollutants. Toxicology 60: 15–25 (1990).